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Contribution of particulates and pH on cowbirds' (*Molothrus ater*) avoidance of grain treated with agricultural lime

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Abstract

Agricultural lime used as a grain coating can be repellent to graniverous birds. However, whether repellency is achieved depends upon the method of preparation. The primary mechanism for mediating repellency is pH. Cowbirds avoid seed coated with agricultural lime (5% wt/wt) when the pH exceeds 12.3. A second underlying component mediating repellency exits that is based on avoidance of particulates. If the particulate seed coating consists of particles sized ~ 63–150 μm, and has a pH of 11.4 or less, the repellent potency is about half that observed for raw unprocessed lime. Together, these data help explain emerging conflicting reports on the efficacy of agricultural lime as a bird-repellent. Finally, short-term data on food and water intake and energy balance suggest that periodic intake of agricultural lime does not adversely affect birds. © 1998 Elsevier Science B.V.

Keywords: Lime; Aversion; Repellent; Cowbird; *Molothrus ater*

1. Introduction

Birds can cause significant agricultural losses (Wywialowski, 1994, 1996). As a consequence, considerable efforts, both lethal and nonlethal, have been employed to

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minimize crop damage attributable to birds (Hygnstrom et al., 1994). However, birds also can be a valuable asset in the control of insects in agricultural settings (Hintz and Dyer, 1970; Mott et al., 1972). Therefore, economical methods to limit the impact of birds on crops without reducing populations would be of great value, especially because a single species may help control insects during one part of the year but be an agricultural pest during other times of the year. Low impact bird control strategies are desirable for nonagricultural reasons as well. Public-opinion surveys have identified the preservation of wildlife resources and biodiversity as desired goals (Sanborn and Schmidt, 1995). In addition, regulatory considerations place primary emphasis on nonlethal methods to resolve human-wildlife conflicts. The research and development efforts for nonlethal bird repellents reflects these needs (Mason and Clark, 1992). One area of recent interest has been the potential use of particulate coatings as bird repellents.

Birds will decrease intake of seeds and turf that have been treated with particulates of various compositions. Blackbirds reduce intake of rice seeds coated with bentonite clays, plaster, or Portland cement (Decker and Avery, 1990; Dolbeer and Ickes, 1994). Starlings (*Sturnus vulgaris*) reduce intake of food treated with specific size classes of activated charcoal (Mason and Clark, 1994, 1995; Clark, 1995). Snow geese (*Chen caerulescens*) reduce consumption of turf treated with activated charcoal (Mason and Clark, 1995). Canada geese (*Branta canadensis*) and brown-headed cowbirds (*Molothrus ater*) avoid turf and feed treated with agricultural lime (Belant et al., 1997a,b).

Birds are capable of making discriminations among food based upon textural properties, and will use texture as the basis for dietary selections (Zweers, 1979, 1985; Best and Gionfriddo, 1994). Independent of the sensomotor aspects of food selection, a variety of functional mechanisms have been proposed to explain avoidance of foods treated with particulates. For example, food coatings may be antinutritive (Mason and Clark, 1994). Alternatively, increased coarseness of particulate-coated food may act as an abrasive, causing gastrointestinal irritation if excessive quantities are consumed (Nir et al., 1994). The coarseness of food may change the perception of the potential food particle such that consumption is commensurate with intake of non-nutritive particles, i.e., grit (Clark, 1995). Finally, some of the coating material may render food unpalatable on the basis of chemosensory attributes, i.e., altering pH (Belant et al., 1997b).

The bird repellent properties of agricultural lime, plaster and Portland cement are of interest because factors of coarseness/granularity and pH occur in these materials and it is unknown to what extent the repellent effect of these materials is due to each of these properties. A better understanding of the mechanism also is warranted because recent studies using similar bioassays (cf. Belant et al., 1997b; Cummings et al., in prep.) have yielded conflicting results for the efficacy of agricultural lime as a bird repellent. We undertook this study to better understand what characters of lime are repellent to birds, i.e., granularity and/or pH. We also wished to understand how the slightly different methods of preparation used by Belant et al. (1997b) and Cummings et al. (in prep.) might have influenced the physical characteristics of lime and how these changes affected the feeding behavior of birds.

2. Animals, material and methods

2.1. Animals

We used brown-headed cowbirds as a model avian granivore of agricultural significance. Birds ($N = 80$) were captured in Sandusky, Ohio and transported to the U.S. Department of Agriculture laboratory in Fort Collins, CO for testing.

2.2. Material

The lime used for the study was agricultural dolmitic hydrated lime ($Ca(OH)_2MgO$, CAS 59398-71-3, GenLime Group) with a reported solubility of 0.1 g/100 g (25°C) and a pH of 11.7 at the saturated concentration (25°C).

2.3. Experiment 1: the effect of application method on food consumption

The objective of the first experiment was to directly compare consumption of millet treated with agricultural lime prepared using methods of Belant et al. (1997a,b) and Cummings et al. (in prep.). These two studies yielded different results for efficacy of lime as a repellent.

Twenty-four experimentally naive cowbirds were selected at random, housed individually (30 × 30 × 60 cm), and maintained on a 12:12 L:D light cycle at 19–22°C. Cowbirds were maintained on an ad libitum diet of millet, cracked corn, sunflower seed, meal worms and water. During the pretreatment adaptation period, birds were food deprived at 1700 h. Experimental diet (millet) was provided from 0900–1200 h the next morning, after which time the experimental food cups were removed and the birds were returned to their maintenance diet. This feeding sequence was repeated for four days. Experimental diet consumption was determined gravimetrically. To prevent the inclusion of outliers in the test only birds whose 3-h food intake was within ±2.5 standard deviations units of the mean were included in the study. Three birds were excluded from the study because of abnormally low food consumption of the control ration. These birds showed a history of weight-loss and subsequent post-mortem analysis showed them to have a bacterial infection. The remaining cowbirds were assigned at random to one of 4 groups. A further precondition for the start of the test was similarity for average food consumption among groups for days 3 and 4 of the pretreatment adaptation period. Similarity among groups was verified statistically using a fixed effects, two-factor repeated measures analysis of variance (ANOVA), with consumption as the dependent variable, group assignment as the between subjects effect and day the within subjects (repeated measure) effect (STATISTICA, 1994).

Each group was assigned at random to receive millet treated in one of four ways: dry-mixed, slurry-mixed, filtered solution-soaked, and a negative control. The dry-mixed preparation was formulated by tumble-mixing 5 g of agricultural lime with 95 g of millet. Inspection of the millet indicated particles up to 150 μm adhering to the surface of the millet, although most of the lime did not adhere to the surface of the millet. Rather, it was intermixed with the millet. This method of preparation corresponded to that used by Belant et al. (1997a,b). For the slurry-mixed method, a suspension of agricultural lime in distilled, deionized water (5% wt/wt) was prepared. Next, 20 g of millet was mixed with 100 g of suspension under agitation for 2 h. The slurry was vacuum-filtered and air dried at room temperature (23°C) for 24 h. Inspection of the dried millet showed particles of lime up to 60 μm adhered to the surface of the millet; and the millet had a dusty appearance, indicating the preponderance of smaller particles coating the seeds. This method corresponded to that used by Cummings et al. (in prep.). For the filtered solution-soaked method, the filtered supernatant of a 5% lime suspension was used to soak the millet as was done for the lime slurry-mixed treatment. No visible particles under 10 × magnification were distinguishable, but the dried millet appeared dull compared to millet treated with water indicating a coating of ultra-fine particles. The difference between the slurry-mixed and filtered solution-soaked treatments was primarily a matter of when in the processing the filtering step occurred. In the slurry-mixed method, undissolved lime particles and solute could adhere to millet during drying, where as in the filtered solution-soaked method all particulates > 5 μm were removed prior to treating the millet. Thus, only solute was available for deposition onto the seed-hull during drying. The three treatment methods presumably reflect differences in actual concentration of residue coatings and particulates interspersed with millet despite the similarity of concentration of lime used at the start of each process. It is these differences in processing that presumably served as the basis of the contradictory findings of Belant et al. (1997a,b) and Cummings et al. (in prep.), and provided the rationale for our experiments. The negative control was obtained by soaking millet in water for 2 h followed by 24-h air drying. The pH of processed, dried millet was determined by mixing 5 g of treated millet in 95 ml of deionized water for 2 h, then measuring the pH of a filtered aliquot of the solution.

Birds were food-deprived and presented with experimentally prepared millet using the schedule described for adaptation and tested over four days. Data were analyzed as a fixed effects, 2-factor repeated measures analysis of variance (ANOVA) design with treatment type as a between measures effect and days as the repeated measure. The repeated measures design was used to test for possible learned avoidance of millet as a function of processing method.

2.4. Experiment 2: concentration-response relationship to agricultural lime solutions

The objective of this experiment was to determine the concentration-response relationship of cowbirds to agricultural lime solutions. Testing the response to solutions allowed us to determine the effects of pH on consumption independent of the particulate properties of agricultural lime. Birds are typically insensitive to pH of solutions (Feurst and Kare, 1962), showing indifference to a variety of inorganic acids and bases ranging in pH 2.0–12.0. The pH of a saturated solution (0.1 g/100 g) of dolmitic-hydrated agricultural lime used in the tests is listed as 11.7 at 25°C (MSDS, GenLime Group), suggesting that based upon pH alone, this material should not be repellent to birds. However, laboratory analyses of the lime indicated a saturated solution (0.2 g/100 g) had a pH of 12.4 at 25°C, a value typically avoided by many birds. This information also indicated that the composition of the commercial lime was not exclusively $Ca(OH)_2MgO$.

Twelve cowbirds were selected at random and individually housed and maintained as described in Experiment 1. After adaptation, and as a precondition for testing, baseline consumption of food and water was verified. No birds deviated more than 2.5 standard deviation units from mean food consumption for the maintenance ration. Therefore, no birds were excluded from this test. Five solutions (pH 7.5, 8.5, 9.8, 11.4, and 12.4) of agricultural lime were tested. Dilutions were achieved serially, starting from a filtered saturated stock solution (0.2 g lime/100 g deionized water). Each bird was presented with a single test-solution during a standard 1-bottle, 3-h drinking assay (Clark, 1996) for each of five days. To control for possible carry-over effects (i.e., learning) the order of presentation for solutions across days for each bird was determined randomly. Comparison of intake among test solutions was made using a one-way fixed effects ANOVA. A paired *t*-test (pre- vs. post-test) was used to verify that the overall presentation of lime solutions across days did not affect baseline water intake of individuals.

2.5. Experiment 3: partition of the effects of particles and pH on food treated with lime

The objective of this test was to experimentally eliminate the effect of pH on consumption for millet treated with lime. Experiment 1 showed that the water soluble fraction of lime adhering to millet using the dry-mixed method was 12.4, and that this pH was within the range normally repellent to birds (Feurst and Kare, 1962). To separate the pH and particle effects on the avoidance response, we processed the lime prior to its application to the millet. Processed lime was prepared by washing raw agricultural lime under continuous flow of water (20°C), discarding the soluble components while collecting the relatively insoluble particles using a sieve (> 60 μm). A 0.2 g/100 ml water suspension of material was prepared and the pH was measured as 10.8, a value theoretically not aversive to birds. Following this process step, the residual lime was dried to constant weight and reserved.

Selection of birds, adaptation protocol, and food and water consumption criteria for inclusion in the test followed that outlined in Experiments 1 and 2. Nine cowbirds were selected for testing the three treatment levels: plain millet (negative control), millet coated with unprocessed lime, 5% wt/wt (positive control), and millet coated with processed lime, 5% wt/wt. Each bird was presented with each of the treatments twice in a standard 3-h 1-cup feeding assay over a six-day period, with the order of presentation determined randomly. This presentation schedule was used to minimize possible carry-over effects resulting from repeated presentation of strongly alkali treated food (sensu Experiment 1). Consumption of maintenance food and water were also monitored during the non-test period. This information was a useful indicator of post-ingestional test effects of the food treatment method and as an index of energy balance of birds over the course of the test.

A difference score for the rate of food intake was used as an index of the effect of treatment on post-test food consumption while controlling for individual variation in food consumption. The mean within subject's change in the rate of food intake is defined as:

$$\overline{\Delta R_j} = \Sigma\left(R'_{i,j} - R''_{i,j}\right)/N_j \qquad (1)$$

where, $R''_{i,j}$ is the rate of millet consumption (g h^{-1}) during the 3-h test period for the ith bird presented with the jth treatment, $R'_{i,j}$ is the rate of maintenance food consumption (g h^{-1}) during the 5-h post-test period for the ith bird presented with the jth treatment during the preceding 3-h test period, and N_j is the number of birds receiving the jth treatment. Pattern analysis on this index allowed us to determine whether consumption or avoidance of treated food influenced energy balance.

3. Results

3.1. Experiment 1: the effect of application method on food consumption

Food consumption varied as a function of the method of treating the millet and day of test ($F = 2.56$; $df = 9, 60$; $P = 0.015$). Inspection of the post-hoc test (Tukey's Honestly Significant Difference, HSD) indicated that consumption of food remained constant as a function of time for birds presented with millet prepared as a lime slurry, soaked with filtered lime solution, and soaked in water (the control). However, there was an indication that consumption of food treated as a dry-mix increased on the fourth day of presentation ($P < 0.05$). Because the interaction effect was confined to a change in consumption pattern for a single treatment method on the last day of the test, we felt that inspection of the main effect of treatment ($F = 37.36$; $df = 3, 20$; $P < 0.001$) best characterized the general response of the birds to preparation method (Fig. 1). Relative to birds fed an untreated diet of millet (the control), cowbirds presented with millet treated with lime using the dry-mix method decreased food intake by 67% ($P < 0.05$). Cowbirds presented with millet prepared as slurry (5% wt lime/wt millet) did not show consumption different from that seen in the control ($P > 0.05$). Cowbirds presented with

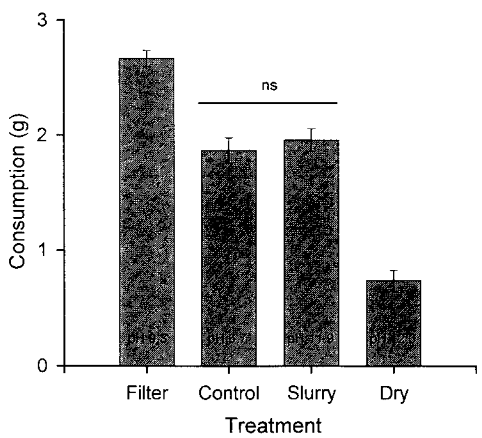

Fig. 1. Mean (± SEM, vertical capped bars) millet consumption by cowbirds during a 3-h test period as a function of treatment method (see Methods: Section 2.3). The horizontal lines indicate post-hoc comparisons with associated probabilities (ns is $P > 0.05$). The pH of water extractable components of the millet coating is indicated within the shaded bars.

millet soaked in a filtered supersaturated lime solution (5% wt/wt) preferred the treated seed relative to the control ($P < 0.05$). Food intake was not linearly related to pH. The pH values of aqueous extractions of formulated food were 6.7 for the control, 9.3 for the filtered formulation, 11.9 for the slurry formulation, and 12.3 for the dry-mixed formulation. The different formulations were visually discernable to the human observer: control millet appeared glossy; the filtered formulation appeared dull, but otherwise untextured; millet in the slurry formulation appeared to have a finely powdered patina of lime, and; millet in the dry-mixed formulation was coated with obvious granular particles of lime. This experiment shows that the method of preparation influences palatability of millet, and that a dry-mixed preparation is repellent, though consumption is not statistically distinguishable from zero intake ($P > 0.05$).

3.2. Experiment 2: the effects of pH on fluid consumption

Fluid intake of cowbirds varied as a function of pH ($F = 11.33$; $df = 4, 55$; $P < 0.001$). Intake by cowbirds presented with fluids ranging in pH 7.5–11.4 was similar (Fig. 2). Intake of fluid at pH 12.4 was suppressed relative to solutions of lower pH ($P < 0.05$). Fluid intake for each of the pH solutions within the range of 7.5–11.4 were similar to pre- and post-test baseline water intake (Fig. 2), suggesting that these solutions had no effect on fluid consumption by cowbirds. The post-test tap water intake (cf. pre-test intake) was not affected by lime solutions presented over the course of five consecutive days ($t = -0.45$, $df = 18$, $P = 0.656$). Food consumption did not vary across days or treatment during the test. Together, these data indicate that cowbirds avoid the same range of pH for basic solutions as other birds, and that ingestion of fluids with pH's of 7.5–11.4 had no short-term effect on normal water or food consumption (circa 5 days).

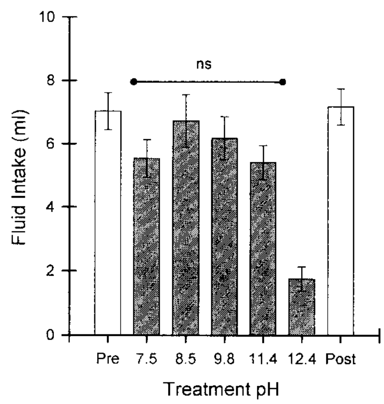

Fig. 2. Mean (grey bars ± SEM, vertical capped bars) fluid consumption by cowbirds during a 3-h test as a function of pH of the solution. The horizontal lines indicate post-hoc comparisons with associated probabilities (ns is $P > 0.05$). The open bars are provided for reference and depict tap water consumption during a 3-h period of the test subjects a day prior to and a day after the five-day test period.

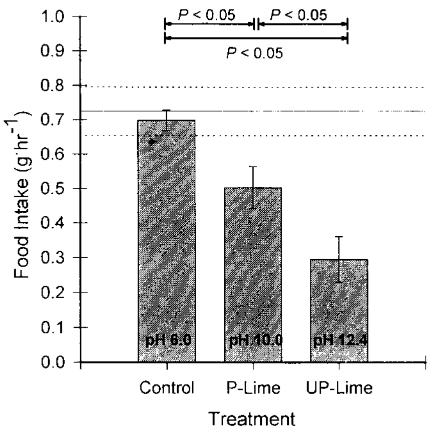

Fig. 3. Mean (±SEM, vertical capped bars) rate of feed intake by cowbirds as a function of treatment method. For reference, the horizontal solid line depicts the mean pretreatment rate of food consumption (±SEM, horizontal dotted lines). The pH of water extractable components of the millet coating is indicated within the shaded bars.

3.3. Experiment 3: partition of the effects of particles and pH on food treated with lime

Intake of untreated millet in this test was similar to that observed in Experiment 1 (0.7 g h^{-1} $\pm$ 0.03 SEM and 0.7 g h^{-1} $\pm$ 0.05 SEM, respectively), as was the rate of intake for millet treated with agricultural lime using the dry-mix method (0.3 g h^{-1} $\pm$ 0.05 SEM and 0.3 g h^{-1} $\pm$ 0.05 SEM, respectively). The method of treatment influenced the rate of millet intake ($F = 12.75$; $df = 2, 50$; $P < 0.001$). Consumption of

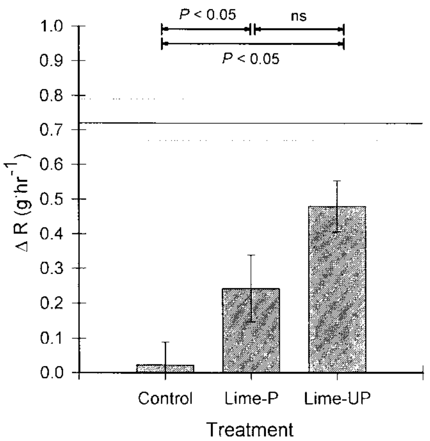

Fig. 4. Mean (±SEM, vertical capped bars) change in the rate of millet intake (ΔR) by cowbirds as a function of treatment type. The control is untreated millet, P-Lime is millet coated with processed lime, and UP-Lime is millet coated with unprocessed lime. The solid horizontal lines depict associated probabilities for group differences based on the post-hoc test.

millet treated with both processed and unprocessed lime was reduced relative to the control (Fig. 3). Millet coated with unprocessed lime reduced food intake relative to millet treated with processed lime.

ΔR differed among the treatment groups ($F = 10.88$; $df = 2, 50$; $P < 0.001$). Cowbirds presented with untreated millet during the 3-h test period had a ΔR similar to zero, indicating that the rate of food intake was constant between the two observation periods. Cowbirds presented with both types of lime-treated millet had larger ΔR's than the control ($P < 0.05$). The magnitude of the ΔR's was directly and inversely related to the magnitude of the suppressive effect of the treatment type on feed consumption (cf. Figs. 3 and 4). This compensation response in feeding behavior allowed the birds to maintain body weight throughout the study (pre- vs. post-study body weight, $t = -1.96$, $df = 8$, $P = 0.085$) and suggests that, within the time-frame of the study, periodic ingestion of lime-treated feed had no post-ingestional consequences on feeding behavior or change in body weight.

4. Discussion

Belant et al. (1997a,b) and Cummings et al. (in prep.) reached opposite conclusions about the efficacy of agricultural lime as an avian repellent. On the basis of Experiment 1, it seems both studies have claim to legitimacy. The method of preparation is important to the effect observed. Belant et al. (1997a,b) prepared food in a manner consistent with the dry-mixed method of preparation in Experiment 1. This preparation method results in food avoidance by birds. The Cummings et al. study prepared food in a manner consistent with the slurry method of preparation and application in Experiment 1. In this case birds were indifferent to the treatment effect. Interestingly, by introducing a third preparation method we were able to generate yet another outcome: cowbirds actually preferred the treated food relative to the controls. For dry-mix preparations the food has both pH and possible particulate coating properties that might explain avoidance of the treated food. The pH of an aqueous extract of the treated food is 12.4, a value generally repellent to birds (Feurst and Kare, 1962) and specifically repellent to cowbirds (cf. Experiment 2). However, a certain fraction of the dolmitic lime (0.125% wt/wt) falls within the size class of non water soluble particles shown to be effective as starling repellents (~ 150 μm). While this represents a low concentration, it is feasible that avoidance of treated food may in part be due to particulate effects (sensu Clark, 1995). Food treated as a slurry did not have obvious particles adhering to the surface of the millet. Rather, the coating of the millet appeared to be a fine powder with particle sizes of < 60 μm; a size class of particle that was not repellent to starlings (Clark, 1995). The process of solubilizing the lime while exposing millet to the solution also affected the nature of the powdered coating. Aqueous extracts of the treated food resulted in pH's of 9.4, levels well within the palatability tolerance of most birds, and specifically cowbirds. Therefore, this method of preparation destroyed both pH and particulate attributes of agricultural lime that might otherwise contribute to repellency, hence the observed similarity to the intake levels observed for controls and the 'no effect' reported by Cummings et al. The outcome resulting from the third preparation method is enigmatic. However, one possible mechanism suggests itself. Filtering the test solution prior to treating millet removes all particulates from the process and the post-treatment wash is only pH 9.4. The resulting treated food has a dull texture compared to the untreated millet, but with no obvious powdered residue. Soaking in basic solution may alter the flavour characteristics of the millet which is slightly acidic. Granivores show clear preferences to millet varieties that correlates with tannic acid content (Cheeke and Shull, 1985). The hypothesized positive effects of reduced tannic acid may be offset by particulate and basicity effects resulting from the other two treatment methods.

Cowbirds avoided solutions prepared from dolmitic lime of pH 12.4, but were indifferent to solutions ranging in pH of 7.5–11.4 (Experiment 2). Thus, in the absence of particles, cowbirds showed the typical avian tolerance for basic solutions. Exposure to these basic solutions did not appear to affect baseline water consumption or general health of the birds.

Dolmitic lime is composed of a variety of basic materials with varying pH potential in saturated solutions and different water solubilities. When the readily soluble components of the lime were separated from the crude lime, the remaining components represented about 2.5% of the parent material. At saturation, the pH of this residue was pH 10.8 (at 22°C, 24 h). This pH was not sufficiently high to be aversive to cowbirds. However, a 5% concentration of the granular components applied to feed did result in a 29% reduction in the rate of food intake relative to the control. The dry-mixed treatment, containing both pH and granular effects, resulted in a 67% reduction in the rate of food intake relative to the control. If the contribution of pH and particulates were additive we might assume that the contribution of these factors was approximately equal, i.e., the relative contribution of pH to the avoidance response might be a (67% − 29% =) 38% reduction in consumption of treated food relative to the control. However, because the drinking experiments also yielded a 70% decrease in fluid intake at pH 12.4 relative to controls, and this presentation format does not include a particulate effect, we interpret the results to mean that pH 12.4 is the more salient cue for avoidance responding (Experiment 3), overshadowing the effect of the particulates. Thus, we conclude that the repellent effects of a pH treatment of at least 12.4 is about twice as effective as a 5% particulate coating composed of the least soluble components of dolmitic lime within the size range of 63–150 μm.

We are encouraged that birds presented with the liquid or solid forms of the repellent millet showed no signs of carryover effect after consuming material treated with lime. Birds compensated fluid and food intake to maintain water and weight balance, suggesting that homeostatic regulatory mechanisms remained intact.

In summary, these results provide clear evidence that the method of application must be attended to, otherwise the same starting materials can yield the range of potential outcomes. Furthermore, these results indicate that the efficacy of properly prepared lime resides in its pH properties, yet if the proper adhesives are applied, long after the more basic constituents of agricultural lime are washed away, a residual repellency is possible due to particulate effects. The potency of this effect is expected to be half the level as attributable to pH-based avoidance. Extrapolation into application strategies may prove useful into extending the life expectancy of this bird aversion strategy after a single application. Pursuing this line of investigation would have impact for the implementation of a cost-effective, humane, environmentally safe method for reducing bird-agricultural conflicts.

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